Effects of Biocontrol Agents on Plant Growth

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Soilborne root diseases frequently limit the growth and survival of plants propagated in nurseries as well as field-grown crops The first level of defense against such diseases has been the application of chemical pesticides marketed for the control of root diseases. The number and availability of such chemicals, however, has dwindled in recent years because of human health risk and the high cost of registration and re-registration. This has forced the search for viable alternatives, including improved cultural practices and biological controls. A few biocontrol agents are available to nurserymen, but most have been developed for use in food crop production, and little is known about their efficacy in nurseries My research program is dedicated to developing improved strategies for finding and characterizing effective biocontrol agents for application in nurseries. This report is to describe some of the effects of the biocontrol agents on plant growth discovered during this research

BIOLOGICAL CONTROL OF PLANT PATHOGENS

Biological control of plant pathogens, in this case root pathogens, is the use of one or more biological processes to lower inoculum density of the pathogen or reduce its disease-producing activities (Baker and Cook, 1974). Biological control of root diseases is usually the result of the activities of bacteria, actinomycetes, or fungi living and functioning on or near roots in the rhizosphere soil. These microorganisms may be resident in the soil or medium before planting or be introduced at or after planting. They may inhibit root pathogens by antibiosis (production of antibiotic chemicals), by parasitism (direct attack and killing of pathogen hyphae or spores), or by competing with the pathogen for space or nutrients, sometimes by producing chemicals such as siderophores which bind nutrients (such as iron) needed by the pathogen for its disease-causing activities. Microorganisms that suppress fungal root pathogens are everywhere in soil and organic substances, but their numbers may be insufficient to completely suppress pathogens at the time and place where initial infections occur. The strategy of biocontrol research is to find effective antagonists and apply them in high numbers at the potential infection site before pathogen ingress.

FINDING CANDIDATE ANTAGONISTS

Antagonistic bacteria and fungi are present everywhere. We have developed strategies for finding and characterizing these organisms using principles that are fundamentally logical and that have support from some successful examples of biocontrol (Linderman et al., 1983). We have isolated bacteria and actinomycetes from soils or potting media components (peats, composts, etc.) by dilution plating soil solutions (treated first at 50C for 15 min to eliminate all but hardy spore-forming bacteria or actinomycetes) on weak nutrient media and overspraying with spores of test pathogens, such as *Thielaviopsis basicola* or *Cylindrocladium* *scoparium*. Bacterial colonies that show a zone of inhibition of the pathogen are isolated and further tested on several media, at different temperatures, and against several other pathogens including species of *Phytophthora*, *Pythium*, *Rhizoctonia*, *Verticillium*, and *Fusarium*. Bacteria that show promise throughout these tests are studied further to characterize their mode of activity and their efficacy in greenhouse and field tests, applied singly or in combinations with other bacteria or with mycorrhizal fungi. Our studies indicate that the number and inhibitory capacity of antagonists increases in rhizosphere soil, especially in the presence of mycorrhizae (Linderman and Marlow, unpublished results; Meyer and Linderman, 1986).

CHARACTERIZING CANDIDATE ANTAGONISTS

In the course of evaluating candidate antagonists against root pathogens, we have discovered that many bacteria have more than one mechanism of inhibiting fungal pathogens. Sometimes the same bacterium can produce specific antibiotics, Fe-chelating siderophores, and volatile inhibitors, one or all of which can contribute to the suppression of root pathogens. The discovery that many rhizobacteria produce volatile inhibitors, given an appropriate substrate, is a new finding. We have identified the volatile as ammonia gas (NH_3) (Linderman and Marlow, 1992a), and have shown its effectiveness in inhibiting many root pathogens, especially *Phytophthora* and *Pythium* We have developed seedling assays to test candidate antagonists against the black root rot pathogen, *T. basicola*, and the widespread root pathogen, *Phytophthora cinnamomi*. A relatively low proportion of candidate antagonists identified from *in vitro* tests show activity in preventing seedling disease and the degree of protection varies from complete to low. Nearly all the candidate bacteria we have tested, however, show remarkable capacity to enhance plant growth in the absence of the known pathogens. It is this unexpected benefit that is the point of this report.

PLANT GROWTH ENHANCEMENT BY BACTERIAL ANTAGONISTS

We have observed plant growth enhancement in the absence of known pathogens since 1981 (Linderman and Malajczuk, unpublished results), but were unsure of the significance until more reports appeared in the literature (Broadbent et al., 1977, Burr and Caesar, 1984; Chanway and Holl, 1992) and we continued to make such observations in recent studies (Linderman and Marlow, 1992b). In all these studies, we have inoculated plants, usually seedlings, with a suspension of cells of candidate bacteria. In some cases, we have incubated the bacteria on the plant roots for some time before challenging the plants with the root pathogen. In other cases, we inoculated a group of plants with bacteria alone to compare growth with that on plants also challenged with the pathogen In either case, several weeks after inoculating with the bacteria, we observed improved growth compared to the non-inoculated controls (Table 1). There seems no doubt these bacteria grow on the roots of the test plants and produce some metabolite therein that stimulates growth beyond that of non-inoculated control plants. There is often considerable variation in the degree of response by replicate plants given the same treatment, and also some apparent specificity between bacteria and plant species. Undoubtedly, the growth conditions also influence the magnitude of response (Schroth and Becker, 1990). The challenge is to identify the mechanism of activity and to develop the technology to exploit this phenomenon in the propagation of plants.

Table 1. Effects of inoculation with bacterial antagonists on total weight and number of flowers of petunia plants grown for 8 weeks under greenhouse conditions (unpublished data of Linderman and Marlow).

Antagonist treatment	Total plant weight (g)	Number of flowers
9921	3.60 a	5.70 abc
B8	3.53 a	5.60 abc
9620	3.52 a	5.20 abc
9684	3.44 ab	5.50 abc
9645	3.34 abcd	4.40 bcde
6109	3.31 abcd	5.10 abcd
J51	3.30 abcd	6.40 ab
9691	3.29 abcd	5.70 abc
9952	3.28 abcd	7.00 a
9623	3.18 bcd	4.60 bcd
9938	3.14 bcd	4.50 bcde
MS	3.13 bcde	3.80 cde
K18	3.03 cde	2.90 de
Control	2.82 e	2.30 e

* Values followed by the same letter are not significantly different

MECHANISMS OF PLANT GROWTH ENHANCEMENT BY BACTERIAL ANTAGONISTS

Several mechanisms for plant growth enhancement by bacterial antagonists have been proposed in the literature:

- Suppression of deleterious microbes that produce toxins that limit plant growth—their suppression by the inoculated bacterium allows plants to grow closer to their genetic potential,
- Production by the bacteria of growth regulating (phytohormonal) substances that directly stimulate plant growth,
- Increased availability of nutrients that may be limiting plant growth, and
- Induced changes in the microbial composition of the rhizosphere that favor growth-stimulating microbes. The mechanism involved remains in question and is the subject of on-going studies.

CONCLUSIONS

Our results clearly support the contention that antagonistic rhizobacteria, by some means, do influence plant growth. If one were to try to manage this rhizosphere phenomenon, early inoculation of plant propagules (seedlings or rooted cuttings) appears to be necessary. Until the mechanisms of activity are known, it will be difficult to predict potential benefits from inoculation. Perhaps inoculation with combinations of bacteria or combinations with mycorrhizal fungi will come closest to simulating natural conditions of the mycorrhizosphere.

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